Vol. 16 No. 7

July, 1950

THE BOTANICAL REVIEW

Interpreting Botanical Progress

DECENNIAL SUPPLEMENTARY REVIEW ISSUE

Phototropism. II ..... Arthur W. Galston 361

The Flower Constancy of Bees ..... Verne Grant 379

FUTURE CONTENTS
ON INSIDE BACK COVER

Published Monthly except August and September at
111 E. Chestnut St., Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at Lancaster, Pennsylvania, under the Act of March 3, 1879.

THE BOTANICAL REVIEW

Founded in 1935 by
HENRY ALLAN GLEASON and EDMUND H. FULLING
Managed, edited and published at
The New York Botanical Garden
by
EDMUND H. FULLING

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All correspondence should be addressed to THE BOTANICAL REVIEW, at 111 E. Chestnut St., Lancaster, Pa., or Fordham P. O., New York, N. Y.

THE BOTANICAL REVIEW

VOL. XVI JULY, 1950 No. 7

PHOTOTROPISM. II [1]

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INTRODUCTION

In the decade which has elapsed since the appearance of J. van Overbeek's review (53), relatively few investigators have concerned themselves directly with the question of phototropism. This is probably at least partially due to the feeling among plant physiologists that the phenomenon of phototropism is reasonably well understood in terms of auxin and differential growth. Despite this feeling, the present reviewer believes that some of the commonly accepted ideas involved in our understanding of phototropism may be subject to considerable reinterpretation. This paper will therefore be presented in two parts, a review of the new literature and a reconsideration of some of the accepted concepts of phototropism.

Before turning to the body of this paper it would seem desirable to summarize in a paragraph the facts and theories regarding phototropism as presented in the previous review. Phototropism is the curvature of a plant organ elicited by a unilateral light stimulus, and is generally brought about by a difference in growth rates on the illuminated and shaded sides of the organ. In the *Avena* coleoptile and a few other plant organs these different growth rates have been shown to be correlated with and presumably caused by different concentrations of auxin. Recognized ways in which light may influence auxin include inactivation, a directed migration towards the shaded side of the organ, and alteration of rate of production. Blue light is most effective phototropically and is presumably absorbed by a sensitizing pigment, generally assumed to be a carotinoid. It has been held that only auxin-*a* produces differential effects in light and dark, indoleacetic acid (IAA) acting the same under both conditions. The magni-

[1] Supplement to article in The Botanical Review **5**: 655–681. 1939.

tude of the curvature response is dependent on the amount of light energy impinging on the tissue, irrespective of its distribution in time. In stems small amounts of energy generally produce positive curvatures (toward the light), and higher amounts may produce negative curvatures (away from the light). In the *Avena* coleoptile there are two separate growth reactions, one due to absorption of small amounts of light in the extreme tip, the other due to absorption of larger amounts of energy in the base. The pigment systems involved in these reactions are possibly different. The mechanism of phototropic bending in *Phycomyces* sporangiophores and other fungal organs can not be interpreted in terms of auxin, since no auxin requirement for growth of these organs has ever been demonstrated.

As will be made clear in the subsequent discussion, some of the statements in the paragraph above require extensive revision.

RECENT LITERATURE

LIGHT AND AUXIN. Stewart and Went (50) studied the light stability of the auxin in *Avena* coleoptiles. They found the ether-extractable auxin to be partially destroyed by light inside the coleoptile but stable to light after extraction. This difference in behavior in vivo and in vitro is presumably due to the presence of sensitizing pigments in the coleoptile. Light was found to be without effect on the diffusible auxin of the coleoptile inside or outside the coleoptile. Subsequently Went (57) was able to show that tropistic response of decapitated *Avena* coleoptile is correlated not with extractable but with diffusible auxin. Since Stewart and Went found no destruction of diffusible auxin by light, we must conclude that light acts by affecting not the total amount of auxin but only the relative amounts on the illuminated and darkened sides of the coleoptile.

Oppenoorth (39, 40) investigated the effect of various intensities and qualities of light on the ether-extractable auxin of dark and light halves of unilaterally illuminated *Avena* coleoptiles. He obtained evidence for photoinactivation of auxin in the regions of the first positive curvature and second positive curvature. With exposure to higher quantities of radiant energy, auxin synthesis was increased on the illuminated as opposed to the darkened side. Although he could obtain no convincing evidence for lateral trans-

port of auxin in phototropism, Oppenoorth believes it to occur on the basis of experiments on geotropism and an experiment of Boysen-Jensen's which we shall discuss later. In this regard it is interesting to note that Burkholder and Johnston (10) failed to find evidence favoring lateral transport, although they too were able to demonstrate auxin photoinactivation.

Oppenoorth (40, p. 537) disagrees with the view expressed by Blaauw (4) that phototropism is merely a special case of growth inhibition by light, the so-called "light-growth reaction". He found that when auxin inactivation could be demonstrated, it occurred equally on the two sides of the coleoptile and therefore could not lead to differential growth. However, if one accepts the view of Went (57) that diffusible auxins and not extractable auxins are related to tropistic response, then Oppenoorth's evidence, based on extractable auxin, is not relevant.

Yamane (59) covered half of the horizontal intact leaves of *Fatsia japonica* with tinfoil and then exposed the leaves to 400–3900 lux[2]. In cases where the leaves showed positive phototropism, the ratio of auxin in the dark side to auxin in the light side was 1.53; when the leaves were negatively phototropic this ratio was 0.47, and when they were non-responsive the ratio was 0.97. Thus it would seem that auxin changes parallel and possibly are responsible for the movement of these leaves.

Kögl, Erxleben and Koningsberger (33) have reported that auxin-*a* is relatively light-stable, but that its lactone is easily inactivated by ultraviolet light. Although these workers previously (31) had found auxins *a* and *b* not to absorb ultraviolet light of wavelength longer than 2500 Å, they now (33) report an absorption peak at approximately 2900 Å, which they attribute to some unknown photochemical decomposition product of auxin-*a*-lactone. It is interesting that pure IAA has an ultraviolet absorption peak at about 2800 Å and an absorption spectrum almost identical with that of Kögl's hypothetical decomposition product of auxin-*a*-lactone. Another surprising finding is that approximately 3×10^6 molecules of auxin-*a*-lactone are inactivated by the absorption of one quantum of light. Although such high quantum yields could theoretically result from a light-initiated chain reaction, the

[2] One lux = one lumen incident per square meter, or one meter-candle. 10.76 lux = one foot-candle.

value cited is so extraordinarily high for reactions in solution as to be absolutely unique. Perhaps the compound being photoinactivated is already in an unstable condition, so that the decomposition does not give an accurate picture of the true quantum yield.

In corroboration of the work of Schuringa (45), Kögl and coworkers (30) found acid conditions to favor photoinactivation of auxin-*a*-lactone. Kögl and Verkaaik (35) noted about 96% of the auxin of *Phycomyces* sporangiophores to be destroyed by acid and to have a molecular weight (by the diffusion technique) of about 175. This is clearly IAA. The remaining 4% was sensitive to alkali and had a molecular weight of about 330, thus satisfying the requirements for auxin-*a*. Reference is also made to an unpublished experiment of Koningsberger and Verkaaik who applied *Phycomyces* auxin to twice decapitated *Avena* coleoptile and obtained regeneration of phototropic sensitivity and greater auxin destruction on the illuminated side. The auxin obtained from various fungi has a molecular weight (by diffusion) of about 175 and thus is presumably IAA. Notwithstanding this fact and because of their belief that indoleacetic acid can not be operative in light-growth reactions, Kögl and Verkaaik attribute this light growth response to destruction of auxin-*a* via its lactone. However, as will be evident from the subsequent discussion, IAA may also be involved in light growth reactions.

The evidence that only auxin-*a* may be involved in light growth responses was summarized in the previous review (53) on pages 667 and 676. However, Schneider (43) with *Avena* first internode sections, and Galston and Hand (26) with etiolated pea epicotyl sections have shown that light inhibition of growth (the "light-growth reaction") can also occur with IAA, α-naphthalene acetic acid and 2, 4-D as the auxin. Since IAA can participate in the "light-growth reaction", its participation in phototropic curvature is also possible.

Wildman and Bonner (58) have investigated the nature of the native auxin of *Avena* coleoptile. They were able to show that the enzyme which converts tryptophan to IAA is most concentrated in the tip of the coleoptile where auxin is known to be produced. The cold ether-extractable auxin is also almost entirely confined to the tip and was shown to be IAA by the Salkowski colorimetric technique. The diffusible auxin was collected and

found to give a positive Salkowski reaction and to resemble IAA in its diffusion constant, provided that it is first subjected to ether extraction. The authors conclude that the bulk, if not all, of the native auxin of *Avena* coleoptile is IAA. This would also imply that IAA must be involved in the light-growth reactions of *Avena* coleoptile.

PIGMENTS. Kögl and Schuringa (34) have reported that auxin-*a*-lactone, which is normally inactivated only by ultraviolet light, may be inactivated by visible light in vitro, provided one of several carotinoids is present as a sensitizing pigment. They regard this as the system operative in vivo, based also on their belief that auxin-*a* is the native auxin of higher plants.

Galston (23) has shown that IAA may be photoöxidized by blue light in vitro in the presence of riboflavin as a sensitizer. The action spectrum for this reaction (25) follows closely the absorption spectrum of the pigment. If riboflavin is added to the medium in which etiolated pea epicotyl sections are being grown, then severe growth inhibition occurs in the light but not in the dark. If IAA is mixed with a brei of etiolated pea epicotyls, the action spectrum for photodestruction of the IAA is almost identical with that of the IAA-riboflavin reaction. This definitely establishes riboflavin as a major receptor pigment in the destruction of auxin by plant tissue. The quantum yield for the IAA-riboflavin reaction is approximately 0.7 molecule of IAA disappearing per quantum of blue light absorbed.

Riboflavin is abundant throughout the *Avena* coleoptile, occurring uniformly to the extent of about 30 γ per gram dry weight (25). The diffusible auxin is fairly light-stable outside the coleoptile, but when incorporated into an agar block containing 2 γ per cc. of riboflavin, it is rapidly photoinactivated. The rate of inactivation is similar to the rate of photoinactivation of pure IAA under identical conditions. This is taken as evidence that riboflavin could be the sensitizing pigment and IAA the auxin involved in the phototropic response. The action spectrum for the first positive curvature of *Avena* coleoptiles has some points of resemblance with the absorption spectra of both β-carotene and riboflavin, being in general closer to the carotene curve. It is, however, considered by Galston and Baker (25) that in vivo action spectra are not sufficiently precise to distinguish between two pig-

ments whose absorption spectra are as similar as β-carotene and riboflavin. On the basis of the spectral data of Haig (29), they conclude that the "base reaction" probably involves riboflavin as a receptor pigment and auxin destruction as the mechanism; the "tip reaction" probably involves a carotinoid receptor and either destruction, lateral migration or interference with synthesis of auxin.

Oppenoorth (40), although he subscribes to the view that a carotinoid is the photoreceptor in phototropism, obtained a total pigment extract of *Avena* coleoptiles which showed a single absorption peak at about 4450 Å (p. 310). This is precisely the absorption peak of riboflavin, and the data should therefore not be used as evidence in favor of the carotinoid theory. Bünning (9), in the third paper of his series on phototropism and carotinoids, obtains a single-peaked action spectrum for phototropism in *Pilobolus* and *Phycomyces*, as had Castle (12) several years before. He explains the lack of a double peak (found in the absorption spectrum of β-carotene) in terms of differential absorption of different wavelengths on the near and far sides of the coleoptile, assuming that at $\lambda = 4500$ Å 10% of the light is absorbed on the near side. It would seem equally logical to this reviewer to interpret the single peak as real and as due to absorption by flavin. Since riboflavin is known to sensitize the photoinactivation of tryptophan, histidine, methionine and enzymes as well as of IAA (23, 24), the curvature of these fungal sporangiophores need not be interpreted in terms of auxin. Bünning also shows that the distribution of carotene crystals parallels sensitivity to light in the *Avena* coleoptile, although the pigment is deficient in the extreme tip, which is most sensitive to light. The existence of a gradient for carotene and the lack of such a gradient for riboflavin may be considered as support for the carotene theory.

Bünning confirms a previous report by Atkins (1) that green plants respond phototropically to red light. Atkins had found this for green *Avena* seedlings as well as for green plants of *Lepidium* and *Celosia;* Bünning confirms it with *Sinapis alba* and *Helianthus annuus*. It it interesting to note that this phenomenon had previously been reported by Zollikofer (60) and by Bachmann and Bergann (2) but had not received sufficient attention. Flint (21) finds red light ineffective on *Pilobolus* and Italian rye grass, the

former in opposition to the results of Parr (41). If it is true that red light produces phototropic curvature, then some pigment other than carotene or riboflavin must be involved. Chlorophyll is a possible receptor in this region, as are various other tetrapyrroles. Manten (*37b*) has noted the phototropic response of the blue-green alga *Tolypothrix* to blue light to be enhanced by orange light which by itself is ineffective phototropically. He concludes that β-carotene is the photo-receptor for phototropism and that the effect of orange light is mediated through photosynthesis. This explanation might also apply to the observations cited above in which red light was found to effect phototropic curvature.

EFFECTS OF LIGHT ON THE PHYSICAL APPARATUS OF THE CELL. Bottelier (6) demonstrated that four to eight minutes after illumination of an *Avena* coleoptile, the rate of protoplasmic streaming was depressed, the magnitude of the effect being related to the intensity of the light employed. As with phototropism, blue is the most effective wavelength. If the wavelength employed was 4360 Å, the maximal inhibition of streaming was produced by 200 ergs/cm.^{2}, which produces a strong first positive curvature. If the intensity is raised to 800 ergs/cm.^{2}, streaming is actually accelerated. Bottelier suggests that this effect may be important in phototropism in that a decrease in the rate of streaming would decrease the transport of auxin from the tip to the growing cells. Since growth of the cells is limited by auxin, this light interference with auxin transport would lead to differential growth on the two sides of the coleoptile, accounting for the curvature towards the light. Unfortunately it is not entirely clear that auxin transport occurs as a result of protoplasmic streaming, so that the major assumption of Bottelier is still unproved.

Gessner (*27*) found that unilaterally illuminated *Helianthus* hypocotyls showed a decreased plastic extensibility on the lighted side. *Lupinus luteus*, which shows no phototropic curvature, does not display this effect. This author concludes that the effect of light on the cell wall properties may account for observed phototropic curvature. Bünning reports a similar observation for *Pilobolus* curving towards light. Brauner (8) found that blue light caused a great decrease in the turgor of the pulvinal cells of *Phaseolus multiflorus* and thought that the effect might account for the curvature. Red light was also effective, causing him to

conjecture that chlorophylls, as well as carotinoids, are involved as photoreceptors.

With regard to electrical effects, Clark (15, 16) found no easily detectable relation between electrical polarity and auxin transport. For instance, the electrical polarity of a segment of coleoptile can be altered by gravity or light, but the polarity of auxin transport remains unaltered. In contrast with these results, Schrank (44–47) finds a direct relationship between electrical and curvature responses of the *Avena* coleoptile. The side of the coleoptile stimulated by light becomes electrically negative. Since auxin migrates as the anion, this polarization should presumably result in diversion of auxin to the shaded side, with a resulting curvature towards the light. Application of 10 μ amperes of current anywhere on the coleoptile results in a transverse polarization and bending. The curvature is at first towards the positive side, later towards the negative side.

Using 10 MCS of light and 5 μ amperes of current, each of which produces a half-maximal curvature when used alone, Schrank found current to antagonize the light effect when the positive pole is on the shaded side and to augment the light effect when the positive pole is on the illuminated side. This presumably establishes a common pathway and common limiting factor to the two processes. One may conclude, therefore, that changes in bioelectric potentials are associated with phototropic curvature, perhaps in a causal connection. Du Buy and Olson (19) have analyzed the data of Clark (15, 16) and have concluded that instead of showing no correlation between transport and applied current, they show that these two factors are related. This would bring Clark's experiment into agreement with those of Schrank. In view of the convincing nature of Schrank's recent experiments, such an interpretation would seem reasonable.

Wassink and Bouman (55) have analyzed the original data of Blaauw in an attempt to determine how many quanta of light are essential for initiation of phototropic bending in *Phycomyces* and in *Avena*. They assume that if K quanta are required for a reaction to occur, then the chance of the reaction occurring is proportional to the average quantum content of the light flash, according to Poisson's formula. Therefore, if the per cent of curving *Phycomyces* sporangiophores is plotted against the log of the average number of incident quanta, an S-shaped curve results.

The steepness of slope of this curve is a function of the number of quanta required for the reaction to occur.

The plot for *Phycomyces* indicates that absorption of one quantum may initiate bending; for *Avena* the figure is much higher. If this analysis is correct, it would imply that in *Phycomyces* a "chain reaction" is initiated by absorption of the quantum. This chain reaction might involve the auxin photoinactivation described by Kögl and co-workers (33), in which 3×10^6 molecules of auxin are destroyed per absorbed quantum. The main difficulty with such a hypothesis lies in the fact that auxin is not known to be essential for the growth of *Phycomyces*.

Van Overbeek (53, p. 662) states that the Weber-Fechner law holds for phototropism in only a few special cases. However, Pringsheim (42) finds it to hold quite well for the reaction threshold, and Fuller and Thuente (22) find both the Bunsen-Roscoe and Weber-Fechner laws to be followed by all plants tested. Filzer (20) determined the minimal light causing curvature, using both unilateral and bilateral illumination. In the latter situation 17 times as much light is required for curvature. This fact is of interest in view of the widespread use of opposing lights in determining action spectra for phototropism. Burkholder (10) found phototropic curvature to be independent of the angle of incidence of the light.

PHOTOTROPISM IN ROOTS. Naundorf (38) showed that the primary roots of *Helianthus annuus* are negatively phototropic, curving up to 40°. The tip is the most perceptive region, decapitated roots not responding. Replacement of the tip by agar containing IAA gave no curvature, indicating that the tip has other functions in the curvature besides that of auxin supply. These other functions are presumably related to light perception.

Excised roots were also found to respond, although their curvature was less than that of intact roots. Root tips produce auxin, more being produced in the light than in the dark. Growth is also greater in illuminated than in darkened cultures. In a unilaterally illuminated root, the light side produces more auxin than the dark side. Blue light is most effective in producing curvature and in increasing the auxin content of the illuminated side. Segelitz (49) has also reported that light increases the auxin content of isolated roots.

From these data it is clear that phototropism in these roots

involves the auxin production mechanism. Since roots are known to be inhibited by very low concentrations of exogenously applied auxins, it would be logical to expect a positive curvature if light increases auxin synthesis, as it apparently does. The fact that negative curvatures result from the higher auxin levels in light indicates that the endogenous auxin level is sufficiently low that auxin is still limiting to growth. Thus a light-induced increase in auxin synthesis results in a growth acceleration on the near side and a negative curvature.

Dassek (17) finds the rhizoids of various liverworts to be phototropically responsive, the direction of the response to light depending on the refractive index of the medium in which they are exposed. Thus they are negatively phototropic in air or water, positively phototropic in paraffin oil. This effect had previously been obtained and satisfactorily explained by Castle (13).

MISCELLANEOUS OBSERVATIONS. De Ropp (18) noticed that soaking the grain before excision of the embryo increases growth and tropistic response of Petkus winter rye. This is presumably not an effect on the phototropic mechanism itself but rather on growth, probably by way of supplying growth stimulatory substances from the endosperm.

Langham (37) attributed the erect or prostrate habits of certain plants to their positive phototropism at low light intensities and negative phototropism at high light intensities. Experimentally the behavior of his plants seemed to support this hypothesis, since they were erect in darkness and prostrate in bright light.

Chakrabarty (14) reported that the sporophore of *Agaricus polyporus* is positively phototropic during its period of active growth and negatively phototropic after formation of the apical knobs. The reason for this change-over is unknown.

A REEXAMINATION OF SOME ACCEPTED CONCEPTS OF PHOTOTROPISM

IS CAROTENE THE PHOTORECEPTOR[3]? The evidence leading to the view that carotene is the receptor pigment in phototropism is threefold:

[3] Bandurski and Galston (unpublished) have recently found that the carotinoidless coleoptiles of an albino mutant of *Zea* are able to respond to a phototropic stimulus almost as well as the wild-type strain from which they were derived. Since the mutant contains the normal amount of riboflavin, this experiment is strong support for the view that riboflavin, not carotene, is the photoreceptor for phototropism.

a). Carotenes may be demonstrated to occur in the receptor regions of phototropically sensitive organs (9).

b). Carotenes may sensitize the photoinactivation of auxin-*a* in vitro (34).

c). The "action spectrum" for the phototropic response corresponds reasonably well with the absorption spectrum for a carotinoid (30).

This would be convincing evidence were it not also true that the same three statements may be made about another yellow pigment, riboflavin. This pigment occurs in abundance in the coleoptile (25), will sensitize the photodestruction of IAA (23), and has an absorption spectrum which corresponds well with several action-spectra for phototropism (25). In general it may be said that action spectra for the phototropic curvature of *Pilobolus* and *Phycomyces* (9, 12), and that for the "base reaction" of *Avena* coleoptile (29), are closer to the absorption spectrum for riboflavin than to that of a carotinoid. However, the action spectrum for the "tip reaction" (25, 30) seems more closely related to a carotinoid than to riboflavin.

The question then arises: How accurate is an action spectrum in the determination of the photoreceptor pigment? Blum (5, pp. 56–60) discusses this matter in some detail:

"Assuming the system to obey Beer's law at all concentrations, it should be possible to calculate the absorption of any given thickness of solution from the absorption or extinction coefficient. However, it is usually impossible to know the concentration, let alone the thickness of the material after it has been introduced into a given biological system where it is to act as a photosensitizer, so it would seem impossible to make a fair comparison of the absorption spectrum of a dye with the action spectrum for a biological system sensitized by that dye".

When one adds to this difficulty the possible complicating effects of pH, screening pigments and adsorption-orientation effects on light absorption by the pigment in vivo, it should be clear than an action spectrum is not sufficiently precise to distinguish between two pigments whose absorption spectra are as similar as are those of riboflavin and β-carotene. Even the criterion of one peak vs. two peaks is not entirely valid, since a flavin-flavoprotein mixture may have a two-peaked absorption curve (25), and a carotinoid may yield a one-peak action spectrum (9).

Loofbourow (*37a*), discussing the significance of action spectra, concludes:

"In view of these many assumptions, and of further difficulties in the biological aspects of the experiments, it may seem surprising that action spectra have yielded any useful information. Fortunately, if one is aware of the perturbing factors, one can hold them within reasonable limits in many instances, so that useful interpretations of the data are possible. . . . Probably the chief pitfall to be avoided is the assumption, apparently not uncommon, that because the method involves careful measurements with elaborate physical equipment it must necessarily yield meaningful information".

The lack of interest in riboflavin as a receptor pigment for phototropism is probably an accident of chronology. The first precise action spectra for phototropism (30) were obtained at a time when riboflavin had just been discovered and was not generally available for experimentation. Thus early suggestions regarding the similarity between action spectra for phototropism and absorption spectra of carotinoids deflected the attention of subsequent investigators from other possible photoreceptor pigments. Had the flavins been generally known prior to 1934, they might very well have received greater consideration in this connection.

DOES LATERAL TRANSPORT OF AUXIN OCCUR UNDER THE INFLUENCE OF LIGHT? The most definitive evidence for light-induced lateral transport of auxin came from the experiments of Went (56). He showed that if the diffusible auxin content of an unilluminated *Avena* coleoptile tip be regarded as 100, then in the first half hour after illumination it is only 72, in the second half hour it is 88, and in the third half hour it is 87. If a tip is unilaterally illuminated with 1000 MCS of light, the diffusible auxin released by the illuminated half in about 84 minutes is 27, and from the shaded half 57. (The control half is here taken as 50.) By a subsequent experiment in which tips were unilaterally exposed to 100 MCS of light he obtained the following results:

Time after illumination	Curvature in degrees produced by diffusible auxin		
	Light side	Dark side	Sum
1st 75 min.	6.8 ± 0.4	9.6 ± 0.8	16.4
2nd 75 min.	1.8 ± 0.8	15.0 ± 1.0	16.8

Because the distribution of auxin between light and dark sides was altered without any significant change in the total diffusible auxin present, Went concluded that there had been a lateral migration of the auxin.

Another interpretation of these data is possible. It has been shown by van Overbeek (54) that much more diffusible auxin is released from coleoptile tips over an extended period of time than can be extracted at any one time. This implies that the release of diffusible auxin is the result of its continuing synthesis in the tip. It is therefore possible to hypothecate the following mechanism: (*a*) the total quantity of auxin produced by the tip is limited by the amount of auxin precursor coming up the coleoptile; (*b*) the enzymatic auxin-producing apparatus on the "light" side is partially inactivated by light; (*c*) less of the auxin precursor which appears at the tip will then be converted to auxin on the illuminated side; (*d*) the unconverted precursor will then randomly diffuse to the "dark" side, where it will be converted to auxin. Such a mechanism would account for constancy of total auxin production, together with difference in distribution between illuminated and darkened sides, without invoking a light-induced transverse migration of auxin. The fact that riboflavin (and presumably other pigments) is known to sensitize the photoinactivation of various enzymes (24) would lend credence to this view.

The data of van Overbeek (51, 52) have also been conventionally interpreted in terms of a light-induced transverse auxin migration. Using *Raphanus* seedlings, this investigator was first able to show that symmetrical illumination decreased the growth response to auxin but did not influence the quantity of auxin transported through hypocotyl cylinders. Unilateral illumination of the tips caused less auxin to diffuse from the lighted than from the shaded side. The same was true if the tip were removed and replaced by an auxin-containing agar block. Finally, if an auxin-containing agar block were placed asymmetrically over the lighted side, more auxin could still be obtained by diffusion from the darkened half. From these experiments it is quite clear that lateral migration of auxin may occur. Yet the question remains: Is the lateral migration caused by the light? It is certainly equally possible to account for all these data on the basis that auxin moves laterally, uninfluenced by light, but that differential photo-inactivation occurs on the illuminated and shaded sides. This obscure

point could be resolved by an experiment in which balance sheets for auxin in darkened and illuminated tissues are carefully compared.

It is not the purpose of this reviewer to press for the acceptance of this alternative view, but merely to point out that a light-induced lateral transport of auxin has not yet been unequivocally demonstrated. The best evidence for stimulus-induced lateral transport of auxin comes from experiments on geotropism, where the phenomenon seems reasonably well established. The single most convincing experiment in the field of phototropism is probably that of Boysen-Jensen (7) who found that insertion of a physical barrier in the middle of the *Avena* tip prevented phototropic curvature if the barrier was perpendicular to the direction of the light beam, but not if it was parallel to it. The present reviewer has been unable to confirm this experiment (unpublished), but even confirmation would not prove that it is auxin and not precursor which is moving laterally.

Also worthy of note is the experiment of Laibach (36) who found that auxin applied subsequent to illumination increased the curvature due to light. Since this obviously could not be due to photoinactivation of the added auxin, he suggested that the auxin had migrated to the shaded side under the influence of a light-induced polarity. If this is the true explanation, then possibly the effect is caused by the light-induced decrease in cytoplasmic streaming on the near side (6) with a subsequent lateral transfer due to local accumulation of the auxin. We must conclude that, although certain experiments suggest a light-induced transverse migration of auxin, this point has by no means been proved.

CAN LIGHT-GROWTH RESPONSES BE INTERPRETED ONLY IN TERMS OF AUXIN-A? Van Overbeek (52) conducted experiments which led him to the conclusion that if auxin-*a* is supplied to tissue, light will decrease its effectiveness ("light growth reaction"), but that if IAA is supplied to the tissue, light has no effect on its action. The *Avena* experiment was conducted as follows. Coleoptiles were twice decapitated, and then an agar block placed on the top asymmetrically, as in the *Avena* test. One set of plants was illuminated symmetrically and another set kept in the dark room. If IAA was the auxin in the block, no difference in curvature occurred as a result of the light treatment, but if auxin-*a* was used, light de-

creased the resultant curvature. Van Overbeek mentions, however, that since no pure auxin-*a* was available, he was forced to utilize coarse corn meal as his source of this product. He merely soaked a plain agar block in an aqueous corn meal extract for a few hours and then used it as his source of auxin-*a*, assuming this to be the auxin present because the work of Kögl, Erxleben and Haagen-Smit (32) had shown auxin-*a* to be present in corn oil. Yet we now know that corn endosperm contains large quantities of IAA (3) and also adequate quantities of sensitizing pigment, such as riboflavin and carotinoids. In view of the known flavin-sensitized photoöxidation of IAA (23) it is possible to interpret van Overbeek's results solely in terms of pigment-sensitized photoinactivation of IAA in the agar block. The section growth experiments of Schneider (43) and of Galston and Hand (26) further reinforce the view that light-growth responses may be obtained with IAA as the auxin.

It must be mentioned that van Overbeek obtained similar results with pure auxin-*a* from Kögl's laboratory, as did Koningsberger and Verkaaik (35*a*). If this product was actually devoid of any trace of photosensitizer, the result supports van Overbeek's thesis. Obviously this crucial experiment needs to be repeated, and it is hoped that pure auxin-*a* will once again become available for experimentation so that a definite decision on this question may be made.

SUMMARY

The major revisions which we must make in our ideas concerning phototropism are the following:

a) Perception of blue light by phototropically reactive plant parts may be mediated by one or more of several pigments, all of whose absorption spectra agree reasonably well with the action spectrum for phototropism. These pigments include various carotinoids and riboflavin-containing compounds.

b) Lateral transport of auxin under the influence of light, long accepted as a mechanism explaining the auxin changes which accompany phototropic bending, has not been unequivocally demonstrated. The data could equally well be interpreted in terms of a disturbance of a substrate-limited auxin-producing mechanism at the apex of the coleoptile and other plant organs.

c) Indoleacetic acid can participate in light-growth reactions by

virtue of its rapid photoinactivation in the presence of the proper sensitizing pigments.

d) Photoinactivation of auxin may be readily demonstrated in vitro and in vivo, but the relation of auxin photoinactivation to phototropism is still not clear. The effect of light on auxin levels of tissues may be mediated not only by a photoinactivation mechanism but also by a stimulation or inhibition of the auxin-producing process, as well as through effects on auxin migration.

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THE FLOWER CONSTANCY OF BEES

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INTRODUCTION

The flower constancy of the honey-bee was first accurately described by Aristotle (2) in the following terms: "On each expedition the bee does not fly from a flower of one kind to a flower of another, but flies from one violet, say, to another violet, and never meddles with another flower until it has got back to the hive". The phenomenon was well known to the 19th century floral biologists, Charles Darwin, Herman Müller and their colleagues (5, 6, 18, 21, 37, 53, 54). It was subsequently investigated by several students of insect behavior at the turn of the century, particularly by Felix Plateau, Gaston Bonnier, August Forel and Karl von Frisch (9, 26, 27, 29, 61). More recently the bearing of bee constancy on certain practical aspects of agriculture, such as problems of orchard management (10, 11, 13, 15, 16, 51), production of forage crops (40) and contamination of seed crops (4, 20), has come to the fore.

The flower-visiting behavior of the honey-bee, which is a domesticated animal, has thus been the subject of numerous studies during this century and the last. One of the purposes of the following review is to summarize this scattered information for botanists. The flower-visiting habits of wild bees are not so well known as those of the honey-bee. An attempt has nevertheless been made to summarize the principal published observations on flower constancy in wild bees. These observations indicate that wild bees possess an instinct of flower constancy similar in general to that of honey-bees. The writer will undertake, finally, to consider some of the possible consequences of the phenomenon of flower constancy in the evolution of the angiosperms.

THE PHENOMENON OF FLOWER CONSTANCY

A division of labor exists among field bees of *Apis mellifica* in that certain workers carry water, others gather pollen, nectar or propolis (resin from bark and buds), and still others search for new sources of food (9). The instinct of a given individual to

remain constant to a special occupation is quite rigid, moreover, and is maintained over a period of days (57). Only pollen- or nectar-gathering bees, therefore, will have an opportunity to manifest the phenomenon of flower constancy, and in the discussion which follows we shall be concerned with the flower-visiting workers alone.

OBSERVATIONS OF FIELD BEES. The contention that pollen-gathering honey-bees remain true to one species of plant at a time has been confirmed both by direct observation of bees in the field and by examination of their pollen loads when they return to the hive. The observational evidence accumulated by two English naturalists of the 19th century (6, 18) with respect to the honey-bee and the bumble-bee is summarized in Table 1. The obser-

TABLE 1

PROPORTION OF INTRASPECIFIC TO INTERSPECIFIC VISITS MADE BY HONEYBEES AND BUMBLE-BEES, FROM OBSERVATIONS OF BENNETT AND CHRISTY IN 1883

Genus	Authority	Intraspecific visits, per cent	Interspecific visits, per cent	Number of individuals observed	Number of flowers visited
Apis . .	(18)	100.0	0.0	8	258
	(6)	83.3	16.7	6	+ 45
Bombus	(18)	51.0	49.0	51	+1,660
	(6)	69.7	30.3	33	+ 417

vations of Plateau (61), which were not quantitative, brought that author to the same conclusion, namely, that species of bumble-bees are relatively inconstant, whereas the honey-bee is highly constant, though even she occasionally strays from one kind of flower to another.

Only gradually, by the use of marked bees and other improvements of technique, are we coming to an appreciation of all that is subsumed by this word "constancy". Hermann Müller (53) was the first to discover, by following honey-bees daubed on the thorax with colored paints, that constancy is maintained not only throughout a given foraging flight but also over a period of days. He thus observed one marked bee to return repeatedly during 11 consecutive days, and another during ten days, to the same plant of *Salvia aethiopis* in his garden.

Minderhoud (51) subsequently demonstrated, from observations of marked honey-bees on local patches of *Taraxacum officinale*, *Trifolium repens* and *Reseda odorata*, that, given an abundant supply of flowers of one species, the foraging bee instinctively visits an area about ten meters square. Butler, Jeffree and Kalmus (16) place the diameter of the fixation area at about five yards for patches of *Epilobium angustifolium* and *Echinops sphaerocephalus*. One case has been reported where individual honey-bees returned day after day to one individual plant of a group of three bushes of *Cotoneaster* (17). These observations suggest that the constancy of honey-bees to one kind of flower may be related to their constancy to one locality.

In view of the known heterozygosity of many cultivated plants which are pollinated almost exclusively by honey-bees, the foregoing results on local constancy would appear to explain too much. Butler and his co-workers (13, 15, 16) have, however, extended and clarified the observations of Minderhoud. The situation as described by Butler seems to be as follows. The first flowers to bloom in the spring will have a group of bees fixed on them, and it is at this time that territorial repartition is instituted. Bees from other hives, arriving on the scene after the available local patches have all been pre-empted, will not at first be able to forage as a stable group in the manner of their predecessors, but will wander over the field more or less at random. These wandering bees are responsible for much of the wide cross-pollination within the plant population.

But as a second species of plant comes into flower, it is occupied by the erstwhile wanderers which now likewise become constant. This second source of pasturage, even if it is more abundant than the first crop, does not attract the older bees, and, of course, newcomers will now as before have to serve their term as wanderers before they too can find the means to a constant source of pollen.

As a species of plant approaches the end of its blooming period and the number of flowers dwindles, a brief period of competition for pollen ensues, leading to a new wave of inconstancy. The equilibrium between the bees and their pasture is soon reestablished, however, by the natural death of the older bees. The dwindling crop of pollen attracts no young foragers from the hive; forewarned by a communication from the older workers (30, 56), they turn instead to new and more fertile fields.

Butler, Jeffree and Kalmus (16) rightly note that the local constancy of bees is not without important consequences for the genetics of plant populations. Bee plants, even though they occur in a large continuous population and are self-incompatible, "will still not form a homogeneous interbreeding population but rather an aggregate of small visiting systems in the sense of Wright (1921) which do not exceed a few yards in diameter. In short the local constancy of bees results in a considerable degree of inbreeding in the pollinated plants". It is quite possible, therefore (and the point deserves further attention), that both the heterozygosity of bee plants and the panmixia of their populations are less than has commonly been supposed.

The experience of the British seed trade tends to confirm this view. That trade, faced during World War II with the problem of maintaining uncontaminated the pedigrees of different vegetable plants—turnips, radishes, and the like—while producing them in mass on a crowded island, turned to an experimental study of intervarietal crossing by bees (3). Crane and Mather (20) concluded that if a large mass of flowers were available, bees would confine their visits to a small area. When radishes are grown in quantity, for example, an interval of 300 feet guards against contamination by intervarietal crossing. The 300 feet in this case is a conservative isolation distance. Bateman (3) found that the first 50 feet reduced the contamination to 1%; the next 200 feet in Crane and Mather's study diminished it further only to 0.1%, even in the vicinity of a bee-hive.

The conclusion that the local constancy of honey-bees leads to a restriction of panmixia in many plant populations has also received some unexpected support from the field of plant pathology. The fire-blight pathogen, a bacterium (*Erwinia amylovora*), is transmitted by bees from blossom to blossom of apple and pear. The pathogen thrives best in nectar of low concentration; it does not do well in nectar of high or medium concentration (33). "However, in many cases in which the nectar was at a favorable concentration, little or no infection occurred after contaminated bees had sipped from it. It is, therefore, apparent that other factors besides nectar concentration are important in limiting blossom-blight transmission by bees" (33).

EXAMINATION OF POLLEN LOADS. Some data on the relative frequency of pure and mixed pollen loads in honey-bees and

various wild bees are summarized in Table 2. The data of Brittain and Newton (10) were obtained in Nova Scotia and Quebec, mainly from bees on deciduous fruits; those of Clements and Long (19) were obtained at Pike's Peak, Colorado, from bees on wild plants; and the data of Betts (7) were assembled in England. It was convenient to summarize these data in Table 2 by genus, rather than by species, of bee. It is necessary to remark, therefore, that several of the genera, *Andrena* and *Halictus*

TABLE 2

PROPORTION OF PURE POLLEN LOADS TO MIXED LOADS IN SEVERAL GENERA OF BEES, FROM MICROSCOPICAL EXAMINATION OF THE PELLETS[1]

Genus	Authority	Pure loads, per cent	Mixed loads, per cent	Number of individuals examined
Andrena	(10)	43.5	56.5	180
	(19)	64.3	35.7	28
Apis	(10)	62.0	38.0	274
	(19)	87.5	12.5	32
	(7)	93.2	6.8	3,437
Anthophora...	(19)	20.0	80.0	5
Bombus	(10)	58.8	41.2	85
	(19)	49.0	51.0	102
	(7)	57.1	42.9	14
Halictus	(10)	83.9	16.1	247
	(19)	75.0	25.0	8
Megachile ...	(10)	75.0	25.0	4
	(19)	54.5	45.5	22

[1]Minor discrepancies exist at several points between the figures presented here and those given by the authors in their summaries; however, the above figures were compiled from the published data of the authors cited.

for example, consist of numerous species which differ widely in flower constancy, so that a comparison of such large genera with the single species *Apis mellifica* would be quite unrealistic.

Considering the great variation in species of bees, species of plants, place and time, however, the results are on the whole fairly consistent. Brittain and Newton concluded that the honey-bee is not so constant as most authors have assumed; Betts, and Clements and Long, on the other hand, decided that she maintains a high degree of flower constancy. The species of wild bees belonging to

the genus *Halictus* are the most consistently flower-constant for all sets of data.

The wild bee *Anthophora simillima* makes a poor showing in the sparse data of Clements and Long. It may be worthwhile, therefore, to mention the findings of Linsley and MacSwain with a different species, *A. linsleyi* (39). These investigators, combining field observations with an examination of both pollen loads and cell series, found that *Anthophora linsleyi* in two localities adjacent to the Mojave Desert, California, was collecting pollen only from *Salvia carduacea*. The bees by-passed other plants in flower to obtain the sage pollen, even though this made it necessary for them to fly more than a mile from their nest. From an examination of the cells it was evident that the bees provisioned each cell with a single type of pollen, usually the bright red pollen of *Salvia carduacea;* if a cell was found stocked with more than one kind of pollen, the different types were present in different parts of the cell. Bennett (5) long ago observed that in a field where *Lamium album* and *L. purpureum* were flowering together, *Anthophora retusa* visited only the former species of plant. Since the pollen of that species is yellow and that of *L. purpureum* is red, it was comparatively easy for Bennett to verify his observations by a microscopic examination of the pollen loads.

One may easily obtain an exaggerated notion, from the percentages of mixed loads in Table 2, regarding the frequency of interspecific visits by the bees. A pellet composed, let us say, 99% of pollen from *Geranium caespitosum* and 1% of pollen from *Ranunculus acicularis* and *Potentilla gracilis* does not, statistically speaking, point to a very high degree of inconstancy. Such cases are not rare. From Clements and Long's data it is possible to pick them out. Reanalysis of their data from the standpoint of relative degree of inconstancy leads to the following picture. Of the mixed loads of *Apis mellifica* represented in Table 2, every one was 95–99% pure. Most of the mixed loads of species of *Andrena* were similarly more than 95% pure, but two individual bees carried loads which contained more than 5% of foreign pollen. Over half of the interspecific visits paid by species of bumble-bees resulted in pollen pellets which were at least 95% pure.

Having made these allowances, however, we remain with the fact that a certain amount of flower inconstancy, sometimes more, sometimes less, is a normal ingredient of bee behavior. Why this is so

is clear from Butler's account of the inevitable temporary disproportions between the numbers of individuals of bees and the extent of their pasturage.

THE PSYCHOLOGICAL BASIS OF FLOWER CONSTANCY

Sir John Lubbock's experiments with colored papers provided with drops of honey first demonstrated the existence of a color-sense in bees (47). These early experiments, however, were subsequently swamped out by Plateau's extensive writings. Plateau believed that insects are attracted to flowers neither by color nor by shape, but by odor (59; see also 19 for a complete review of Plateau). After a good deal of printer's ink had been expended on this point, Frisch performed in 1914 a series of experiments that were as crucial as they were clearly conceived (27).

Frisch's method was to arrange a set of small colored papers at random on a table; intermixed with the colored papers were several gray papers, forming a graded series from light to dark gray. The block of gray and colored papers was kept under a piece of plate glass in order to eliminate any possible factor of odor. Small watch glasses were next placed on the plate glass above each of the papers. One of the watch glasses, for example the one on the blue paper, was then filled with sugar solution. Honey-bees from a nearby hive were permitted to come to the table and soon found the syrup in this one watch glass. As often as the bees exhausted the supply of syrup in this watch glass, Frisch replenished it. While the bees were away at the hive, moreover, he frequently changed the arrangement of the colored and gray papers in the block, but always kept the supply of syrup in association with the color blue.

After the bees had been conditioned during two days to come to the blue paper for the syrup in the watch glass upon it, Frisch suddenly rearranged the order of the papers and provided syrup in all of the watch glasses. The bees nevertheless returned directly to the blue paper in its new position and ignored the other watch glasses. Or if all of the watch glasses were removed the bees still hovered over the blue paper, but not over any of the gray ones. Since the bees readily distinguished between blue and every shade of gray placed alongside it, they were not color-blind. Frisch asserted: "Die Biene besitzt Farbensinn!".

On the basis of numerous experiments of this type, Frisch (27)

concluded that the honey-bee does not perceive red as a color but rather as black. In the remaining portions of the spectrum the bee has no fine sense of shades; she confuses every shade of blue, and blue with purple, and she fails to discriminate between green, yellow and orange. The bee, according to Frisch, thus sees only two main colors, blue and yellow, in addition to white and black.

Frisch notes in this connection that multicolored flowers in the European flora consist very largely of combinations of blue and yellow, blue and white, or yellow and white. Frisch also brings up the well known rule that flowers possessing the most elaborate contrivances for cross-pollination by bees are also predominantly blue- or purple-colored, a generalization which is usually "explained" by reference to a Lieblingsfarbe. The bee has no color preferences, according to Frisch, and if blue and purple predominate in bee flowers, it is probably because this color (singular) is precisely the one which, to the bee's eye, contrasts most strongly with the green of the foliage.

It is now known that honey-bees, as well as many other flower-visiting insects (the social bees *Bombus bimaculatus* and *Trigona cressoni parastigma*, the solitary bees *Xenoglossa pruinosa* (on *Cucurbita*) and *Melissodes bimaculata* (on *Convolvulus*), the wasp *Polistes annularis*, and some syrphid-flies), can see ultra-violet light (38, 38*b*, 48, 49). The tropical bee *Trigona cressoni parastigma* was thus able to distinguish between ordinary white cards and white cards that reflected ultra-violet in experiments carried out by Lutz (49). It is significant to note, therefore, that some flowers also reflect ultra-violet, for example, species of *Cucurbita*, *Lilium* and *Primula* (49). These facts suggest that discussions of the relationship between the colors of flowers and the behavior of flower-visiting insects must take into consideration the ultra-violet colors of many flowers.

The color-sense of bees consists, in summary, of the perception of four main color groups: *a*) a yellow group which includes vermillion, orange, yellow and yellow-green; *b*) blue-green; *c*) a blue group which includes blue, purple and violet; and *d*) ultra-violet (38*b*). Bees are color-blind for pure red (27, 38, 38*b*). Though they can distinguish different degrees of brightness as white, gray and black, they do not distinguish different shades within one color group. In these respects bees are similar to most other arthropods that have been investigated (38*a*).

The color-sense of the honey-bee is supplemented by a fine sense of form. Bees quickly recognize and long remember geometrical figures of the sort they encounter in nature, and at the flower they rely more on form and characteristic lines than on color shades (27). Frisch attributes the significance of color combinations in multicolored flowers to the role the patterns play in forming recognition features for bees.

Honey-bees, finally, can distinguish odors well and can remember them for days or even weeks (29). Bees conditioned to the fragrance of acacia visit this alone and are not in the least attracted by the odor of rose or lavender. Bees conditioned to the odor of orange-peel distinguish this from 43 other essential oils and confuse it only with bergamot and cedar oil. Foul smelling odors, however, evoked no response from the bees. Since bees can perceive colors at considerable distances but odors only when close at hand, Frisch has inferred that the role of fragrance is not to attract the pollinating bee, but rather to provide her with a most important means of discrimination once she has found the flower.

This hypothesis demands, of course, that the different kinds of flowers visited by bees should each possess a characteristic odor. Anton Kerner (33*a*, p. 203) has indeed pointed out that closely allied species of plants often have very different odors. He cited in this connection four species of *Daphne*, of which *D. alpina* is vanilla-scented, *D. striata* lilac-scented, *D. philippi* violet-scented, and *D. blagayana* clove-scented. A blind man could distinguish between certain species of *Orchis, Rosa, Syringa* and *Sambucus* by their odors, and in *Viola, Primula* and *Habenaria* one member of a pair of species is strong-scented, while the other has no scent.

The researches of Frisch place us in a position to understand certain older observations on bees. The experimental bouquets devised by the earlier workers (19, 22, 59) did not cause the bees to change from one kind of flower to another because they recognized differences in form. One example will suffice. Detto (22) accustomed some bees to heads of *Rudbeckia laciniata*. He then removed the disks of *Rudbeckia*, substituting disks of the (to his eyes) similar *Heliopsis laevis*. Notwithstanding the fact that bees were independently visiting *Heliopsis* at the time, the bees on *Rudbeckia* merely investigated the new disk florets but did not enter them.

Cases of bees going freely between different color forms of the

same species are frequently recorded. Plateau (60) thus reported *Anthidium manicatum* and *Megachile ericetorum* to pass from pink to blue forms of *Salvia horminum* in his garden. Similarly *Bombus terrestris* crossed white and pink varieties of *Althaea rosea*, and blue and pink varieties of *Delphinium ajacis*. *Apis mellifica* crossed blue, violet, pink and white forms of *Centaurea cyanus*.

Darwin (21, ch. xi) reported similar instances: "Humble and hive-bees are good botanists, for they know that varieties may differ widely in the colour of their flowers and yet belong to the same species. I have repeatedly seen humble-bees flying straight from a plant of the ordinary red *Dictamnus fraxinella* to a white variety; from one to another very differently colored variety of *Delphinium consolida* and of *Primula veris;* from a dark purple to a bright yellow variety of *Viola tricolor;* and with two species of Papaver, from one variety to another which differed much in colour; but in this latter case some of the bees flew indifferently to either species, although passing by other genera, and *thus acted as if the two species were merely varieties*". (Italics of the reviewer).

When closely related subspecies or species of plants, differing by some well marked floral characters and not by mere color as in the foregoing examples, are grown together in a garden, the pollinating honey-bees remain in general on one kind of plant at a time and do not cross-pollinate them. This was the case in garden experiments with three subspecies of *Gilia capitata* (31). One of the subspecies, *Gilia capitata chamissonis*, possesses oval corolla lobes and creosote-scented nectar; the other two subspecies, *G. capitata capitata* and *G. capitata tomentosa*, have linear corolla lobes and sweet-scented nectar. The honey-bees in the garden, while freely circulating between the two linear-lobed subspecies, nevertheless demonstrated considerable constancy to either the oval-lobed or the linear-lobed group of gilias. Observations of the bees at the flowers showed that discrimination, involving positive rejection in the presence of the alien kind of flower, was being exercised, and suggested very strongly that the form of the corolla lobes and/or the odor of the nectar were in fact the determining factors.

When 145 progeny of the oval-lobed subspecies, *G. capitata chamissonis*, were grown the following year, it was found that

these consisted of only 2.1% of hybrids with *G. capitata capitata* but 19.6% of hybrids with *G. capitata tomentosa*. In the absence of any significant difference in compatibility between the cross *G. capitata chamissonis* ♀ × *G. capitata tomentosa*, on the one hand, and *G. capitata chamissonis* ♀ × *G. capitata capitata*, on the other, it may be concluded that the difference between 19.6% and 2.1% contamination reveals a markedly lower degree of flower constancy between the former pair of subspecies as compared with the latter.

The significance of this relatively low degree of constancy between *G. capitata chamissonis* and *G. capitata tomentosa* may consist in the fact that *G. capitata tomentosa*, while possessing linear corolla lobes like *G. capitata capitata*, nevertheless approaches *G. capitata chamissonis* in size and other features of the corolla. The floral differences between *G. capitata chamissonis* and *G. capitata tomentosa* may lie at the threshhold of the powers of discrimination of the bees (see illustrations in 31).

When the completely intercompatible species, *Antirrhinum glutinosum* and *A. majus*, which differ in the size and markings of the corolla, were grown intermixed in a garden and open-pollinated by honey-bees, so little interspecific cross-pollination occurred that in the progeny of *A. glutinosum* only 2.9% contamination from *A. majus* was found (50). In the same progeny of *A. glutinosum*, however, 4.2% of the individuals showed contamination from a group of various hybrid derivatives of *A. glutinosum* × *A. majus*, differing from the species mainly in flower color and growing 400 feet away. These results point once more to the conclusion that perception of similarities in floral parts, much more than propinquity, forms the basis for the flower constancy of bees.

THE ORIGIN OF FLOWER CONSTANCY

Darwin (21) postulated that the instinct of flower constancy was established by natural selection, operating to preserve the bee of superior efficiency. A constant bee could work more quickly than an inconstant one, for, having learned how to insert her proboscis in one flower, her newly formed techniques would suffice for all other flowers of the same species. Plateau (61), disagreeing with this hypothesis, held that the advantage to the bee consisted not so much in saving of time as in saving of muscular fatigue during the transport of pollen loads. It does not appear that Plateau's suggestion is incompatible with that of Darwin. Dr. E. G. Linsley

has pointed out in a communication to the author that natural selection will operate more directly on solitary bees than on such social bees as *Apis mellifica* where the pollen and nectar-collectors are non-reproductive.

Loew (42), viewing the problem in the larger perspective of flower-visiting insects as a whole, also held that flower constancy is adaptive. He distinguished, on the basis of correlated morphological and psychological characteristics, three stages of adaptation of insects to flower visits, as follows.

Allotropous insects are flower visitors which are only slightly adapted for pollination. They lack both structural adaptations and flower constancy. Their movements are irregular, and they feed on a variety of foods besides flowers. (COLEOPTERA: most flower-visiting beetles. DIPTERA: Muscidae, Empidae. HYMENOPTERA: many flower-visiting wasps, i.e., Vespidae, Ichneumonidae.)

Hemitropous insects are flower visitors which are partially adapted for pollination. They possess feebly developed structural adaptations and an intermediate degree of flower constancy. Their movements are on the whole regular and moderately skillful. (COLEOPTERA: a few beetles, i.e., *Nemognatha*. DIPTERA: Conopidae, Syrphidae, Bombyliidae. HYMENOPTERA: some wasps, i.e., Pompilidae, also some short-tongued bees, i.e., *Prosopis, Sphecodes*. LEPIDOPTERA: most flower-visiting butterflies and moths.)

Eutropous insects are flower visitors which are completely adapted for pollination. They possess highly developed structural modifications and habits of flower constancy, which enable them to efficiently rifle flowers and cross-pollinate them. (HYMENOPTERA: most flower-visiting bees. LEPIDOPTERA: Sphingidae.)

With respect to bees, Loew (41) proposed another classification which has had considerable theoretical value. Monotropic bees comprise those species of bees which visit the flowers of only a single species of plant (i.e., species of *Andrena*); oligotropic bees are species that visit a few allied species of plants; and polytropic bees go indifferently to very diverse flowers. Since many bees visit only one species of flower for pollen but many other kinds of flowers for nectar, Robertson (64) introduced the parallel terminology (monolectic, oligolectic and polylectic) to refer specifically to the behavior of the pollen-collecting females. These distinctions apply at the level of species of bee and are related only

indirectly to the flower constancy of individual bees. *Apis mellifica* is a polytropic bee, for example, in that the species as a whole may be found working on unrelated species of plants: yet individual workers, as we have seen, do not often stray from a single species of plant until they have exhausted the supplies of nectar or pollen on it.

The long polemic between Robertson and Lovell on the origin of oligotropism in bees therefore concerns us only indirectly in this review. Robertson (62) believed that the oligotropic habit originated as a result of competition between different species of bees, with natural selection intervening in favor of those species which are the least competitive, viz., facultatively oligotropic, in their food habits. "My view is that the bee fauna is all that the flora will support, that there is constant competition between the bees [read "species"], and that natural selection favors those which are the most diversified, i.e., the least competitive in their food habits". Lovell (45) disagreed with Robertson's premise that competition exists between different species of flower-feeding bees. He held that plants produce nectar far in excess of the bees' requirements. Without attempting to pass final judgment on the correctness of Lovell's assertion that competition for nectar does not exist in nature, it must be admitted that he at least did not establish the absence of competition for pollen.

It seems probable on *a priori* grounds that a necessary precondition for the origin of flower constancy in bees must have been the rise of angiosperms with concealed nectar and pollen. In a world of polypetalous flowers with numerous stamens and freely accessible nectar or pollen it is difficult to imagine the existence of any selective advantage for the confinement of visits to one particular kind of flower, and the inhabitants of these generalized flowers in the modern flora do not possess instincts of constancy (31, 42). The discussion of the origin of flower constancy can not be separated, therefore, from a discussion of the mutual evolution of angiosperms and bees.

FLOWER CONSTANCY AND THE EVOLUTION OF ANGIOSPERMS

Most studies on flower constancy have of necessity been carried out with the honey-bee, an Indomalasian species which has attained world-wide distribution only within historical times, or else with native oligotropic bees on introduced plants. The parallel data for

bees in the wild, where known, however, indicate that they too exhibit a high degree of flower constancy. It is legitimate, therefore, to consider some of the possible consequences of the instinct of flower constancy of wild bees in the evolution of the angiosperms.

We may consider our subject, for convenience, in relation to two fundamental patterns of evolution, namely progressive evolution, or the sustained changes in the characteristics of populations, and species formation, or the segregation of variations into distinct populations (see 66).

PROGRESSIVE EVOLUTION. The primitive *Magnolia* type of flower possesses numerous separate petals with little or no color and numerous separate stamens, and usually lacks well developed nectaries (12). Mesozoic prototypes of these flowers may very well have been pollinated, as many of their modern representatives are, by beetles of little skill and no flower constancy (23, 32*a*). The insect-pollinated angiosperms of Mesozoic times must in any case have been inefficiently pollinated in the absence of any of the more specialized groups of flower-visiting insects during that era.

The consequent failure of seed set would have placed a selective value on any changes in the flowers tending to confine the insect visitors to one kind of flower at a time, for less pollen would then have been wasted on random interspecific visits and the reproductive potential of the plants would have been increased. Mutations causing the petals to grow up as a fused corolla tube around the stamens might have fulfilled this condition. Fewer types of insects would find entrance to a tubular corolla than to an open flower. Those insects which could feed on tubular flowers, furthermore, would encounter less competition for the available supply of nourishment, and so would be favored by the development of habits enabling them to remain constant to such types of flowers. The instinct of flower constancy may thus have been preserved by natural selection, both for its advantage to the plant, which became better pollinated, and for its advantage to the insect, which became better fed.

The results of the screening process exercised by the tubular structure of the corolla are apparent today in the different types of insects which inhabit sympetalous and polypetalous flowers. Müller has estimated that in the Alpine flora of Europe, beetles,

wasps, flies and other unspecialized and inconstant insects, which constitute 86% of the insect species on flowers with open nectar, form but 37% of the visitors to flowers with concealed nectar (55, p. 490).

The fusion of the petals into a tubular corolla, which made it possible for the plant to leave off the production of numerous stamens and to store and conceal nectar at the base of the corolla tube, must have tended to restrict the pollinating visitors to the ancestors of the bees, long-tongued flies, moths and butterflies (8). These groups of insects, which feed on tubular flowers and possess long tongues and habits of flower constancy, appeared in geological history at the beginning of the Tertiary period (see 32*b*). Most, viz. 86%, of the sympetalous families of angiosperms that are known in the fossil record at all likewise made their first appearance in the early part of the Tertiary (see 61*a*). By early Tertiary time, therefore, a course of mutual evolution involving sympetalous flowers and long-tongued and flower-constant insects, among them bees, was well established.

A still more specialized method of cross pollination of plants, was attained with the development of special floral mechanisms accessible only to certain kinds of bees and designed so that the stamens and stigma of the flower would touch the bee in some one spot (8). The success of such bee flowers depended upon a combination of factors, among them the coloration of the flower, the presence of a landing platform for the bees, and the efficient operation of the floral mechanism. Those groups of angiosperms which had entered upon a course of floral evolution in relation to pollination by bees would accordingly have evolved a brightly colored corolla tube with an extended lower lip and a reduced number of specialized stamens.

The development of these floral characters, which are the elements of a successful bee flower, will have occurred repeatedly in different phyletic lines of plants, just as the transition to flower constancy, long tongues and body hairs has occurred repeatedly in different orders of insects. The same combination of floral characters—two-lipped tubular corollas colored blue or yellow and containing sweet-scented nectar, a reduced number of stamens, and a superior ovary composed of several fused carpels—has arisen independently in several different phyletic lines. This com-

bination of characters is present in the more or less distantly related families Labiatae, Verbenaceae, Papilionaceae, Polygalaceae, Scrophulariaceae, Violaceae, Fumariaceae inter alia.

SPECIES FORMATION. The flower constancy of bees, based on the perception by the bees of likenesses and differences in the form, color and odor of flowers, constitutes a mechanism for the isolation of populations of bee plants which differ in floral characters (31). The operation of this instinct of flower constancy has two important consequences in the process of species formation in bee plants.

The principal evolutionary role of the isolating mechanism based on the pollinating behavior of bees is to augment the efficiency of geographical speciation in bee plants (31). It follows that angiosperms which are pollinated by bees will possess a more effective means of evolutionary divergence than angiosperms which are pollinated by inconstant insects (promiscuous plants). More rapid species formation should thus occur in bee plants than in promiscuous plants. A survey of the flora of Southern California, which is an area very rich in endemic bees, did in fact reveal a significantly higher number of species per genus in bee plants than in promiscuous entomophilous angiosperms—5.9 and 3.4, respectively (31).

It also follows from the nature of the isolating mechanism imposed by the flower constancy of bees that bee plants will in general be more differentiated into species on floral characters than promiscuous angiosperms. A survey of the taxonomic characters which have been used in a wide variety of floras and monographs to separate pairs of species in the ultimate divisions of diagnostic keys for several hundred genera, showed that floral characters do indeed predominate in bee plants. Forty per cent of the taxonomic characters between closely related species of bee plants pertain to the petals and other inner floral parts; the corresponding figure in promiscuous entomophilous angiosperms is but 15% (31).

Bee pollination, therefore, has brought about both a convergence in the structural characters of flowers related to the floral mechanism, and a diversification in the form, color and odor of flowers. The latter category of floral characters, upon which the taxonomy of bee plants is to a large extent based, has arisen as the recognition features of bees. Those groups of angiosperms which have

imposed flower constancy on bees and other insects by such developments as the tubular corolla have undergone a diversification of flower forms, colors and odors as a necessary concomitant to the practise of flower-constant pollination (27, 31).

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. E. G. Linsley, of the Division of Entomology, Dr. G. L. Stebbins, of the Division of Genetics, and Dr. L. Constance, of the Department of Botany, University of California, for criticizing the manuscript and making many helpful suggestions.

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